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ANTIMYCOBACTERIAL ACTIVITY OF THE LEAVES EXTRACT OF *Canarium schweinfurthii ENGL*.

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ABSTRACT.
Preliminary screening of the crude extracts of the leaves of *Canarium schweinfurthii* Engl obtained from serial extraction using hexane, ethyl acetate and methanol as solvents was carried out against BCG using the broth dilution technique. The result showed that the hexane, ethyl acetate and methanol extracts have a minimum inhibits concentrations of 1500ug/ml, 800ug/ml and 2500ug/ml respectively. Further fractionation of the ethyl acetate extract on an open column led to isolation of a compound with a minimum inhibitory (MIC) concentration of 250ug/ml. This proved the claimed by the traditional practitioners that the plant is used to manage tuberculosis symptom.

KEY WORDS. Antimycobacterial, *C,schweinfurthii,* Activity, Leaves Extract.

INTRODUCTION.

Tuberculosis (TB) is the leading cause of death worldwide due to single infectious agent that ends fatally in more that 50% of untreated cases (Graham *et al*, 2004). This disease is curable in virtually all cases provided it is properly handle to avoid resistant of the bacterial to a range of anti-TB drugs (Asres *et al*, 2001). The developing countries are worse hit due to low level of hygiene and standard medical infrastructure. The situation in Nigeria is alarming for being rank fourth among the 22 countries that have the highest global burden and have the highest burden in Africa (WHO report, 2007). Resistance to the current drugs being used is on the increase thereby leading to long duration of treatment, hence the need to source for new anti-TB agent.

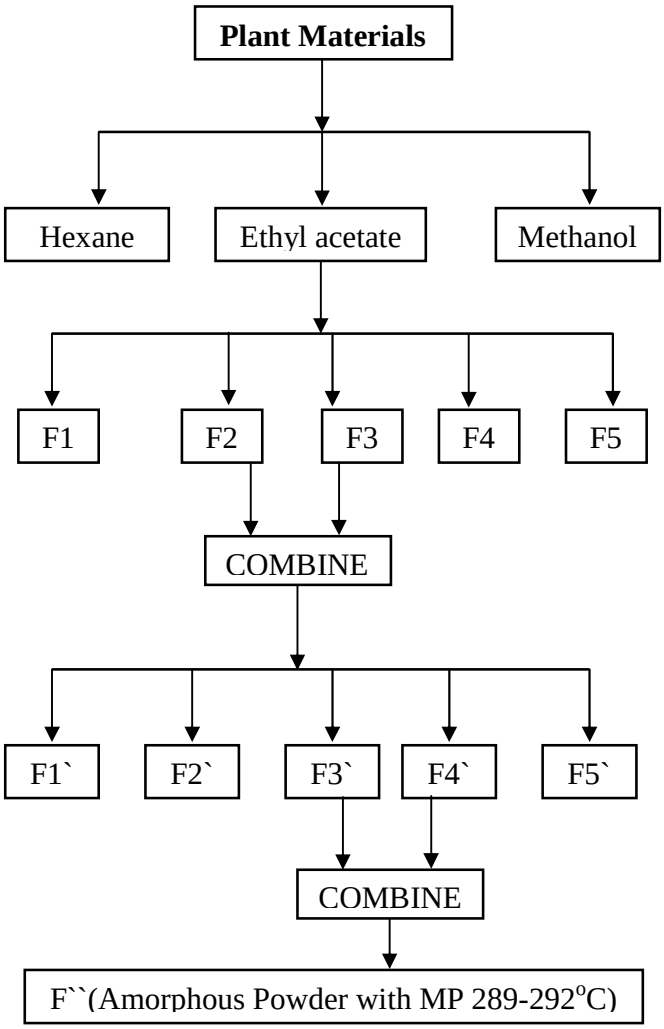
Plants have provided an arsenal of chemicals to survive attacks by a microbial invasion (Martini *et al*, 2004). These chemicals could be of importance in inhibiting the growth of this organism in plants and animals. Literature's showed that Natural products and their derivatives represent more than 50% of the drugs in clinical use with one quarter originating from higher plants (Cragg *et al*, 1997). *Canarium schweinfurthii Engl* is a tree growing in the equatorial forest region of Cameroun, Central Africa and Gabon. The plant also grows in some part of Nigeria as an economic tree. The fruit pulps contain 30% to 50% of oil used to manufacture shampoo and bio-fuel (Obame *et al*, 2007). The oil is used for cooking food and as cream for new born baby in plateau state, Nigeria (verbal communication). The roots stem bark and leaves are used for treating fever, constipation, malaria, diarrhea, sexual infection and rheumatism (Koudou *et al*, 2005). The works of Koudou *et al*, 2005), Agbo *et al,1992) and* Obame *et al*, (2007) show that the oil have some analgesic properties. The biological properties of the plant have long been known and have been used to increase the production and shelf life of food as well as human health (Dongmo *et al*, 2010). The biological properties are most often due to the fractions of essential oils contained in the plants (Hulin et al, 1998). Loius *et al*, 2007 reported on the antioxidant and antimicrobial activity of the oil, the work show that the oil posses both antioxidant and antimicrobial properties. The people of plateau state Nigerian claimed that the leaves are used to treat chronic wound and TB symptom. These inform us to evaluate the plant scientifically against BCG to prove this claimed.

PLANT MATERIAL COLLECTION

Canarium schweinfurthii Engl was collected in Jos south, Plateau state, Nigeria in February 2010. The authentication was done by the ethno botanist of the Department of Medicinal Plants and Traditional Medicine of the National Institutes for Pharmaceutical Research and Development (NIPRD) Abuja. A voucher specimen of the plant has been deposited in the herbarium of the department for features reference.

EXTRACTION AND PURIFICATION OF THE ETHYL ACETATE EXTRACT.

The powdered air-dried leaves (0.5kg) were serially extracted with hexane, ethyl acetate and methanol respectively for 24h with intermittent shaking. The extracts were concentrated on a rotary evaporator at reduce temperature and finally dried completely on a water bath at 40^{o}C. The ethyl acetate extract (10g) was subjected to fractionation using Silica gel (100g, 70 to 230 mesh), eluting with hexane and increased concentration of ethyl acetate to methanol (Fig. 1). The extracts were subjected to phytochemical screening in accordance with the Siddiqui and Ali method (1997).



KEY F =Fraction

Fig I. Flow chart for the extraction and purification of the extract.

BIOASSAY EVALUATION PROCEDURES.

Determination of anti-tubercular activity against *Mycobacterium tuberculosis* ($H_{37}Rv$) was carried out as described by Clifton Barry III laboratory, TRS, NIAID, USA. This was accomplished using the micro well serial dilution method. A 1/10 dilution of the test extract/compound in dimethyl sulfoxides (DMSO) was made in the media; 50µl of media was introduced into wells 2-12. 100µl of the extract/compound was delivered into well 1, 50µl was taken from well 1 and

delivered into well 2 after through mixing 50µl was transferred from well 2 to 3 and the procedure was repeated through to well 12 and from well was 50µl discarded. Thereafter, 50µl of inoculums was added to all wells and it was incubated for 7days. DMSO and ethambutol were use as control and standard drug respectively.

RESULTS AND DISCUSSION.

The crude extracts obtained from the maceration extraction of the leaves showed that methanol had the highest yield of 80g with a minimum inhibitory concentration (MIC) of 2500ug/ml. The yield for the hexane and ethyl acetate extracts were 15g and 10g, with minimum inhibitory concentration of 1500ug/ml and 1000ug/ml respectively (Table1).

The ethyl acetate extracts having the best MIC, due to that it was subjected to open column chromatographic technique using Silica gel (100g, 70 to 230 Mesh) packed in hexane as the mobile phase. The concentration of the hexane was gradually reduced with increase in ethyl acetate to 20% methanol. The fractions sizes of 100ml were collected monitored closely on a Thin layer chromatography (TLC plate) (Silica 254 Marck) and were pulled into ten major fractions as showed in Table 2. The weights of the ten fractions labeled F1 to F10 were noted and 7mg of each of the fractions were screened for their anti-tuberculosis activities. F4 and F5 within the solvent range of 40:60 (hexane: ethyl acetate) were pulled together based on the Thin layer chromatography (TLC) profile and the anti-TB activities. These fractions (F4 & F5) were further fractionated using silica gel (50g. 240 to 400 mesh) eluting with a constant gradient of hexane-ethyl acetate mixture (3:7) resulting in five fractions (F1', F2', F3', F4' and F5'). The combined Fractions F3` and F4` on standing over night gave a creamy powder (22mg), with a melting point of 289-293 ^{o}C (F``) and inhibit the growth of BCG with a MIC of 250µg/ml. Elucidation of the structure of F`` is ongoing.

Also phytochemical screening of the leaf extract of *C.schweinfurthii* reveals the presence of saponins, tannins, glycosides, steroids, carbohydrates and fatty acids. Alkaloid, digitalis glycosides and anthraquinones were not detected. The summary of the result is shown in Table 3 and agree with the work of Ngbede *et al* (2008).

Table 1. Weight of Extracts and MICs Obtained from 0.5kg of the Powdered Leaves.

SOLVENTS USED	YIELDS(g)	MIC (µg/ml)
HEXANE	15	1500
ETHYL ACETATE	10	1000
METHANOL	80	2500

Table 2. Weigh of fractions and MICs Obtained from 10g of the Ethyl acetate on Open column.

FRACTIONS	WEIGH (mg)	MICs (µg/ml)
F1	200	1200
F2	1800	1000
F3	500	700
F4	800	400
F5	1200	350
F6	400	600
F7	500	800
F8	800	850
F9	2000	1500
F10	100	2000

Key F1= Fraction one

Table 3. Results of phytochemical screening on the leaves of *Canarium schweinfurthii Engl.*

Groups	Leaves
Saponins	+ve
Tannins	+ve
Alkaloids	-ve
Glycosides	+ve
Steriods	+ve
Anthraquinones	-ve
Carbohydrates	+ve

Key: +ve = present, -ve = absent.

CONCLUSION

The results showed that the leaf extract of *Canarium schweinfurthii Engl* is active on tubercuclosis causative agent. It is there suggested that the leaves of the plant is evaluated for toxicity and for possible herbal drug formulation, since the leaves is renewable.

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